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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

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INVENTOR(S)

Given Name (first and middle [if any])	Family Name or Surname	Residence (City and either State or Foreign Country)
Kyung-Jin	Yeum	Winchester, MA

Additional inventors are being named on the ____ separately numbered sheets attached hereto

TITLE OF THE INVENTION (500 characters max)

Bioavailability of Carotenoids

Direct all correspondence to: **CORRESPONDENCE ADDRESS**

X	Customer Number:	021125
OR		
	Firm or Individual Name	Nutter, MCClennen & Fish, LLP
	Address	World Trade Center West 155 Seaport Boulevard
	City	Boston
	State	MA
	Zip	02210-2604
	Country	USA
	Telephone	(617) 439-2000
	Fax	(617) 310-9000

ENCLOSED APPLICATION PARTS (*check all that apply*)

X	Specification *Number of Pages*	27		CD(s), Number	
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X	Application Data Sheet. See 37 CFR 1.76				

METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT

X	Applicant claims small entity status. See 37 CFR 1.27.
X	**A check in the amount of $80.00 is enclosed to cover the filing fees.**

Respectfully submitted,

[Page 1 of 1]

SIGNATURE _____

TYPED OR PRINTED NAME Thomas J. Engellenner

TELEPHONE (617) 439-3948

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USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

PROVISIONAL APPLICATION

FOR

UNITED STATES PATENT

Entitled:

BIOAVAILABILITY OF CAROTENOIDS

Inventor:

KYUNG-JIN YEUM

NUTTER McCLENNEN & FISH LLP
World Trade Center West
155 Seaport Boulevard
Boston, MA 02210-2604
Tel: 617-439-2000
Fax: 617-310-9000

Atty. Docket No. 5363-3226

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BIOAVAILABILITY OF CAROTENOIDS

BACKGROUND OF THE INVENTION

Oxidative stress has been implicated in the etiology of chronic diseases such as cancer and cardiovascular disease (Benzié et al *Eur J Nutr* 2000;39:53-61). Numerous epidemiological studies have indicated that diets rich in fruits and vegetables are correlated with a reduced risk of such diseases (Liu et al *Int J Epidemiol* 2001;30:130-135, Greenberg et al JAMA 1996;275:699-703; Gaziano & Hennekens *Ann NY Acad Sci* 1993;691:148-55; Riemersma et al *Lancet* 1991;337:1-5).

Antioxidant nutrients, present in the fruits and vegetables can prevent damage from harmful reactive oxygen species, which are continuously produced in the body during normal cellular functioning. Dietary supplementation with antioxidants can be a part of a defense strategy to minimize oxidative damage in vulnerable population such as the elderly. However, recent clinical studies link high beta-carotene consumption with harmful effects, including a higher incidence of lung cancer in individuals exposed to extraordinary oxidative stress (Werner Siems et al. *FASEB J.* 2002 Aug;16(10):1289-91.) In addition, results from intervention trials indicate that supplemental beta-carotene enhances lung cancer incidence and mortality among smokers (Palozza P et al. *Mol Aspects Med.* 2003 Dec;24(6):353-62).

Accordingly, a need exists for methods of antioxidant supplementation that can rapidly, consistently and effectively protect against DNA damage. In addition, a need exists for a combination of low levels of antioxidants that produce a protective effective effect without harmful side effects.

SUMMARY OF THE INVENTION

The invention is based, in part, on the discovery of the synergistic effect of lutein, beta-carotene, and lycopene in decreasing oxidative damage in human lymphocytes. Methods of decreasing DNA damage through the administration of a carotenoid supplement to a subject are

disclosed. Furthermore, the methods of the invention can be used to protect against certain disorders that arise from oxidative stress and the presence of excess free radicals in a subject.

Accordingly, in one aspect, the invention pertains to a method of decreasing DNA damage through the administration of a combination of carotenoids. The combination of physiological doses of lutein, β-carotene and lycopene have a synergistic effect resulting in a decrease of DNA damage that exceeds that of carotenoids given alone.

In another aspect, the combination of physiological doses of lutein, β-carotene and lycopene changes the antioxidant capacity in the aqueous and lipid compartments of plasma. In yet another aspect, the combination of physiological doses of lutein, β-carotene and lycopene improves DNA response to an oxidative stress. DNA is less susceptible to oxidative damage following supplementation of the mixture of physiological doses of lutein, β-carotene and lycopene.

In yet another aspect, the invention provides a list of genes that are sensitive to dietary carotenoids, or "molecular signature" of carotenoid actions *in vivo*. This list was obtained through gene expression profiles of whole blood samples and quantitative analysis of mRNAs extracted from the whole blood using high density oligonucleotide arrays of ~ 20000 human genes. Functional classification of the differentially expressed genes suggests that genes that regulate immune surveillance are targets of dietary carotenoids. Increased carotenoid concentrations in plasma obtained through dietary supplementations affect transcriptional processes in nucleated blood cells and the hematopoietic cells.

In one embodiment, the combination of carotenoids are given in a single dose. The single dose may be solid, liquid, applied topically or intravenous. In a preferred embodiment, the carotenoids are contained in a solid preparation that can be taken orally (see, for example, US Patent Application No. 09/929,075). In some embodiments, the solid preparation may be combined with a lipophilic component. The combination of carotenoids can also be taken in combination with dietary fat. The solid preparation may, for example, use a permissible oil, such as sesame seed oil, corn oil, cotton seed oil, soybean oil or peanut oil, and esters of medium-

chain plant fatty acids at a concentration of from 0 to 500% by weight, preferably from 10 to 300% by weight, particularly preferably from 20 to 100% by weight, based on the active compounds. The solid preparation may also be taken with a meal containing a sufficient fat content (e.g. greater than 1 gram, preferably greater than 10 g, more preferably greater than 25 g) so that the substantially water immiscible carotenoids can be fully absorbed by the subject. Combining the carotenoid preparation with a lipophilic component increases the antioxidant capacity in the aqueous and lipid compartments of plasma.

DETAILED DESCRIPTION OF THE INVENTION

The methods of the invention can be used to protect against lymphocyte DNA damage and free-radical associated disorders in a subject. The methods of the present invention can be used to increase the antioxidant capacity in one or both of the aqueous and lipid compartments, decrease DNA oxidation, increase gene expression of a panel of genes affected by carotenoids, decrease lipid peroxidation, or increase antioxidant nutrient levels in the circulation. The protective effect of a physiologic dose of a mixed carotenoid supplement, according to the invention, is rapid, consistent and cumulative.

So that the invention is more clearly understood, the following terms are defined:

The term "free radical" as used herein refers to molecules containing at least one unpaired electron. Most molecules contain even numbers of electrons, and their covalent bonds normally consist of shared electron pairs. Cleavage of such bonds produces two separate free radicals, each with an unpaired electron (in addition to any paired electrons). They may be electrically charged or neutral and are highly reactive and usually short-lived. They combine with one another or with atoms that have unpaired electrons. In reactions with intact molecules, they abstract a part to complete their own electronic structure, generating new radicals, which go on to react with other molecules. Such chain reactions are particularly important in decomposition of substances at high temperatures and in polymerization. In the body, oxidized free radicals can damage tissues. Antioxidant may reduce these effects. Heat, ultraviolet light, and ionizing radiation all generate free radicals. Free radicals are generated as a secondary effect of oxidative metabolism. An excess of free radicals can overwhelm the natural protective

enzymes such as superoxide dismutase, catalase, and peroxidase. Free radicals such as hydrogen peroxide (H_2O_2), hydroxyl radical (HO•), singlet oxygen ($^1O_2$), superoxide anion radical ($O•_2^-$), nitric oxide radical (NO•), peroxyl radical (ROO•), peroxynitrite ($ONOO^-$) can be in either the lipid or compartments.

The term "subject" as used herein refers to any living organism in which an immune response is elicited. The term subject includes, but is not limited to, humans, nonhuman primates such as chimpanzees and other apes and monkey species; farm animals such as cattle, sheep, pigs, goats and horses; domestic mammals such as dogs and cats; laboratory animals including rodents such as mice, rats and guinea pigs, and the like. The term does not denote a particular age or sex. Thus, adult and newborn subjects, as well as fetuses, whether male or female, are intended to be covered.

The phrase "free radical associated disorder" as used herein refers to a pathological condition of in a subject that results at least in part from the production of or exposure to free radicals, for example, oxyradicals, or other reactive oxygen species *in vivo*. The term "free radical associated disorder" encompasses pathological states that are recognized in the art as being conditions wherein damage from free radicals is believed to contribute to the pathology of the disease state, or wherein administration of a free radical inhibitor (e.g., desferrioxamine), scavenger (e.g., tocopherol, glutathione), or catalyst (e.g., SOD, catalase) are shown to produce a detectable benefit by decreasing symptoms, increasing survival, or providing other detectable clinical benefits in protecting or preventing the pathological state. Examples of free radical disorders include, but are not limited to, ischemic reperfusion injury, inflammatory diseases, systemic lupus erythematosis, myocardial infarction, stroke, traumatic hemorrhage, spinal cord trauma, Crohn's disease, autoimmune diseases (e.g., rheumatoid arthritis, diabetes), cataract formation, age-related macular degeneration, Alzheimer's disease, uveitis, emphysema, gastric ulcers, oxygen toxicity, neoplasia, undesired cell apoptosis, and radiation sickness. Such diseases can include "apoptosis-related ROS" which refers to reactive oxygen species (e.g., O_2^-) which damage critical cellular components (e.g., lipid peroxidation) in cells stimulated to undergo apoptosis, such apoptosis-related ROS may be formed in a cell in response to an

apoptotic stimulus and/or produced by non-respiratory electron transport chains (i.e., other than ROS produced by oxidative phosphorylation).

The term "oxidative stress" as used herein refers to the level of damage produced by oxygen free radicals in a subject. The level of damage depends on how fast reactive oxygen species are created and then inactivated by antioxidants.

The term "deviation" or "deviate" are used interchangeably herein and refer to a change in the antioxidant activity of a sample. The change can be an increase, decrease, elevation, or depression of antioxidant activity from a known normal value. For example, an increase or decrease of antioxidant activity in the lipid compartment of a sample, the aqueous compartment of a sample, or in both the lipid and aqueous compartment of the sample.

Carotenoids have *in vitro* antioxidant activity at physiological oxygen tensions (Zhang & Omaye, *Toxicol in Vitro* 2001;15:13-24). However, this antioxidant effect has not been conclusively demonstrated in humans (Krinsky NI. *Carotenoids and oxidative stress. In Oxidative stress and aging: Advances in basic science, diagnostics, and intervention.* (Gutler RG, Rodriguez H Eds.) World Scientific Publishing Co., New York (in press)). It should be noted that the metabolism of carotenoids, and possibly their functions, differ *in vivo* among species. Carotenoids can interact with each other during intestinal absorption, metabolism and blood clearance, and individual responses can differ markedly (van den Berg & van Vliet *Am J Clin Nutr* 1998;68:82-89; Paetau et al *Am J Clin Nutr.* 1997;66:1133-1143; Kostic et al *Am J Clin Nutr* 1995;62:604-610; White et al *J Am coll Nutr* 1994;13:665-671.). The present invention describes the antioxidant activity in human blood of a combination of the major carotenoids in fruits and vegetables, such as lutein, β-carotene and lycopene. The synergistic effect of these carotenoids result in a protective effect against free-radical associated disorders and oxidative stress. As shown in the Examples, the methods of this invention is based on the true antioxidant potentials of dietary antioxidants, and the interactions that may take place among these nutrients.

The invention pertains to a method of decreasing DNA damage through the administration of a combination of carotenoids. The combination of physiological doses of lutein, β-carotene and lycopene have a synergistic effect resulting in a decrease of DNA damage that exceeds that of carotenoids given alone. The carotenoid content can range from 0.5 to 20 mg of beta-carotene, from 0.5 to 20 mg of lycopene and 0.5 to 20 mg of lutein, preferably from 1 to 15 mg of beta-carotene, from 1 to 15 mg of lycopene and from 1 to 10 mg of lutein, particularly preferably from 2 to 10 mg of beta-carotene, from 2 to 10 mg of lycopene and from 1 to 5 mg of lutein.

The mixture of carotenoids is given in a single dose. The single dose may be solid, liquid, applied topically or intravenous. In a preferred embodiment, the carotenoids are contained in a solid preparation that can be taken orally (see, for example, US Patent Application No. 09/929,075, Publication No. 2002/0044991). In some embodiments, the solid preparation may be combined with a lipophilic component. The solid preparation may, for example, use a permissible oil, such as sesame seed oil, corn oil, cotton seed oil, soybean oil or peanut oil, and esters of medium-chain plant fatty acids at a concentration of from 0 to 500% by weight, preferably from 10 to 300% by weight, particularly preferably from 20 to 100% by weight, based on the active compounds. The combination of carotenoids can also be taken in combination with dietary fat. The solid preparation can be taken with a meal containing a sufficient fat content (e.g. greater than 1 gram, preferably greater than 10 grams, more preferably greater than 25 grams) so that the substantially water immiscible carotenoids can be fully absorbed by the subject. Combining the carotenoid preparation with a lipophilic component increases the antioxidant capacity in the aqueous and lipid compartments of plasma.

The mixture of physiological doses of lutein, β-carotene and lycopene changes the antioxidant capacity in the aqueous and lipid compartments of plasma. The mixture of physiological doses of lutein, β-carotene and lycopene can also improve DNA response to an oxidative stress. For example, DNA is less susceptible to oxidative damage following supplementation of the mixture of physiological doses of lutein, β-carotene and lycopene. Thus, the methods of the present invention can be used to protect against a free radical associated disorder.

As shown in the Examples, DNA damage in human lymphocytes was decreased following consumption of a combination of carotenoids for 8 weeks. The Examples compare the DNA damage following consumption of individual carotenoids (12 mg of one of lutein, β-carotene or lycopene) to a combination of lutein, β-carotene and lycopene (4 mg each). The combination was shown to produce rapid DNA protection at low doses. The three carotenoids were found to have a synergistic effect. This may be due to the differences in their polarity (i.e., lutein is more polar; lycopene has more conjugation) so that when taken together their functional bioavailability is increased.

Carotenoid Supplement

Carotenoid supplements useful for the present invention can be produced using a number of methods as disclosed in the patent literature for formulating carotenoids. For example, EP-A-0 065 193 and EP-A-0 937 412 describe processes for converting carotenoids into finely divided pulverulent forms. EP-A-0498 824 discloses a process for grinding carotenoids in a protective-colloid-containing aqueous medium and subsequent conversion of this dispersion into a dry powder. EP-A-0 410 236 relates to a process for producing colloidal carotenoid preparations by contacting a suspension of a carotenoid in a high-boiling oil with superheated steam, emulsifying this mixture in an aqueous protective colloid solution and subsequent drying. WO 98/26008 describes a process for producing stable aqueous dispersions and dry powders of xanthophylls. WO 99/48487 describes preparations of carotenoid mixtures in which the carotenoids originate from natural sources. Owing to the high phospholipid content in these preparations, together with a high viscosity of the oily dispersion, the service properties of this formulation are not always satisfactory.

The above-mentioned preparations, when carotenoid mixtures are used, not infrequently encounter problems with stability and bioavailability. In addition, in the case of mixtures having extremely different contents of the individual carotenoids, formation of aggregates among the carotenoids can lead to unwanted inhomogeneous distributions of the active compounds in these preparations. Furthermore, mixtures of dry powders of individual carotenoids also frequently display separation during transport or storage.

In a preferred embodiment, solid preparations of carotenoids is used. The preferred solid preparation of active carotenoid compounds useful for the present invention is suitable for the food sector and animal feed sector or for pharmaceutical and cosmetic applications having a multicore structure, in particular carotenoid-containing dry powders, a process for their production and the use of these solid preparations for producing food supplements and as additive to foods, animal feeds, pharmaceutical and cosmetic preparations is described in US Patent Application No. 09/929,075.

Stable, homogeneous equal distribution of active compounds can be enhanced by administering the compounds in the form of a multicore structure in which at least two cores of a multicore structure have a different chemical composition. The multicore structure is a particle species (secondary particle) having a mean particle size of from 5 to 3000 μm, preferably from 10 to 2500 μm, particularly preferably from 50 to 2000 μm, very particularly preferably from 100 to 1000 μm, in which a further particle species (primary particle), called cores, is embedded in a matrix, the cores having a mean particle size, preferably, of from 0.01 to 1.0 μm, particularly preferably from 0.03 to 0.5 μm, very particularly preferably from 0.05 to 0.2 μm. Examples of such multicore structures are found, inter alia, in U.S. Pat. No. 5,780,056 and in the diagrams described there and in D. Horn and E. Luddecke: "Preparation and characterization of nano-sized carotenoid hydrosols" in Fine Particle Science and Technology, 761-775 [E. Pelizzetti (Ed.), Kluwer Academic Publishers, Netherlands, 1996] and H. Auweter et al., Angew. Chem. Int. Ed. 38 (1999) 5, 2188-91.

The primary particles of the multicore structures are identical in composition, that is to say in the case of a mixture, for example of carotenoids, each core is identical with respect to type and amount of the carotenoid individual components present therein. Unwanted interactions between the active compounds within the multicore structure are prevented or decreased by encapsulation of the individual active compounds, and secondly they permit more flexible organization of the production of user-friendly formulations of active-compound-containing mixtures.

The preferred supplement comprises a mixture of beta-carotene, lycopene and lutein. However, the supplement can contain other active compounds suitable for the food sector and animal nutrition sector or for pharmaceutical and cosmetic applications including, but not limited to the following compounds: Fat-soluble vitamins, for example the K vitamins, vitamin A and derivatives such as vitamin A acetate, vitamin A propionate or vitamin A palmitate, vitamin D_2 and vitamin D_3 and vitamin E and derivatives. Vitamin E in this context is natural or synthetic alpha-, beta-, gamma- or delta-tocopherol, preferably natural or synthetic alpha-tocopherol, or else is tocotrienol. Vitamin E derivatives are, for example, tocopheryl C_1-C_{20}-acyl esters such as tocopheryl acetate or tocopheryl palmitate. Water-soluble vitamins, in particular ascorbic acid and its salts such as sodium ascorbate, and vitamin C derivatives such as sodium, calcium or magnesium ascorbyl 2-monophosphate or calcium ascorbyl 2-polyphosphate, calcium pantothenate, panthenol, vitamin B_1 (thiamine), as hydrochloride, nitrate or pyrophosphate, vitamin B_2 (riboflavin) and its phosphates, vitamin B_6 and salts, vitamin B_{12}, biotin, folic acid and folic acid derivatives such as tetrahydrofolic acid, 5-methyltetrahydrofolic acid, 5-formyltetrahydrofolic acid, nicotinic acid and nicotinamide. Compounds having vitamin character or coenzyme character, for example choline chloride, carnitine, gamma-butyrobetaine, lipoic acid, kreatine, ubiquinones, S-methylmethionine, S-adenosylmethionine. Polyunsaturated fatty acids, for example linleoic acid, linolenic acid, arachidonic acid, eicosapentaenoic acid, docosahexaenoic acid. Food pigments such as curcumin, carmine or chlorophyll. Carotenoids, not only carotenes but also xanthophylls, for example beta-carotene, lycopene, lutein, astaxanthin, zeaxanthin, capsanthin, capsorubin, cryptoxanthin, citranaxanthin, canthaxanthin, bixin, beta-apo-4-carotenal, beta-apo-8-carotenal and beta-apo-8-carotenic esters.

The carotenoids present in the cores can be of either natural or synthetic origin. They generally have a purity of at least 80%, preferably greater than 90%, particularly preferably greater than 95%, very particularly preferably greater than 98%, determined by quantitative HPLC analysis. In the case of carotenoids from natural sources, for example lutein or lycopene, it is possible that these compositions can comprise up to 20% of other carotenoids as "impurities."

A dry powder of this type comprises a multicore structure of secondary particles in which at least three primary particles have a different carotenoid composition, in each case one particle species comprising only beta-carotene, the second lycopene and the third only lutein.

The content of beta-carotene, lycopene and lutein in the inventive dry powders is generally from 0.1 to 50% by weight, preferably from 1 to 35% by weight, particularly preferably from 5 to 25% by weight, very particularly preferably from 8 to 20% by weight, based on the total amount of the formulation.

In the case of the abovementioned ternary combination, the quantitative ratio of the carotenoids present in the dry powder is 1 part of beta-carotene, from 0.02 to 20 parts of lycopene and from 0.02 to 20 parts of lutein, preferably 1 part of .beta.-carotene, from 0.1 to 5 parts of lycopene and from 0.1 to 5 parts of lutein, particularly preferably 1 part of beta-carotene, from 0.2 to 2 parts of lycopene and from 0.1 to 2 parts of lutein, very particularly preferably 1 part of beta-carotene, from 0.3 to 1.2 parts of lycopene and from 0.1 to 0.8 parts of lutein.

In the carotenoid formulations, in particular the abovementioned ternary combination, in addition, the phosphorus content in the formulations is less than 2.0% by weight, advantageously less than 1.0% by weight, preferably less than 0.5% by weight, particularly preferably less than 0.1% by weight, very particularly preferably less than 0.02% by weight, based on the total amount of the mixture of beta-carotene, lycopene and lutein. The low phosphorus content is at the same time associated with a small amount of phospholipids, which improves the service properties of the dry powders, for example the flowability in oily dispersions particularly at low temperatures.

The carotenoid formulations can comprise, in their secondary particles, in addition to the above-described carotenoid-containing cores, other primary particles whose active compounds do not originate from the carotenoid class of substances. These are preferably vitamin-containing primary particles.

The primary particles have a core/shell structure in which the active-compound-containing core is surrounded by a protective colloid. Suitable protective colloids are either electrically charged polymers (polyelectrolytes) or neutral polymers. Typical examples are, inter alia, gelatin, such as beef gelatin, pig gelatin or fish gelatin, starch, dextrin, plant proteins, such as soy proteins, which may be hydrolyzed, pectin, guar gum, xanthan, gum arabic, casein, caseinate or mixtures thereof. However, use may also be made of polyvinyl alcohol, polyvinylpyrrolidone, methyl cellulose, carboxymethyl cellulose, hydroxypropyl cellulose, flake shellac and alginates. For more details see R. A. Morton, Fat Soluble Vitamins, Intern. *Encyclopedia of Food and Nutrition*, Vol. 9, Pergamon Press 1970, pp. 128-131.

Preferred protective colloids are compounds selected from the group consisting of gelatin, such as beef gelatin, pig gelatin and fish gelatin, plant proteins, pectin, casein, caseinate, gum arabic and shellac. Protective colloids which are particularly preferably used are aqueous solutions of gelatin, pectin, casein, caseinate, gum arabic and/or fish gelatin.

To increase the mechanical stability of the dry powder, it is expedient to add to the colloid a plasticizer, such as sugars or sugar alcohols, for example sucrose, glucose, lactose, invert sugar, sorbitol, mannitol or glycerol, or else polymers such as polyvinyl alcohol or polyvinylpyrrolidone. Plasticizers preferably used are sucrose, sorbitol and lactose.

The ratio of protective colloid and plasticizer to active compound is generally chosen so that a solid preparation is obtained which comprises from 0.1 to 50% by weight of at least two active compounds, from 10 to 50% by weight, preferably from 15 to 35% by weight, of a protective colloid and from 20 to 70% by weight, preferably from 30 to 60% by weight, of a plasticizer, all percentages being based on the dry matter of the formulation and the total of the percentages of the individual components being 100%.

To increased the stability of the active compounds to oxidative degradation, it can be advantageous to add from 0 to 10% by weight, preferably from 0.5 to 7.5% by weight, based on the dry matter of the formulation, of one or more stabilizers, such as alpha-tocopherol, tert-butylated hydroxytoluene, tert-butylated hydroxyanisole, ascorbic acid or ethoxyquins.

In addition, emulsifiers can be used, for example ascorbyl palmitate, polyglycerol fatty acid esters, sorbitol fatty acid esters, propylene glycol fatty acid esters or lecithin at a concentration of from 0 to 200% by weight, preferably from 5 to 150% by weight, particularly preferably from 10 to 80% by weight, based on the active compounds used.

In some circumstances it can also be advantageous to use in addition a physiologically permissible oil, for example sesame seed oil, corn oil, cotton seed oil, soybean oil or peanut oil, and esters of medium-chain plant fatty acids at a concentration of from 0 to 500% by weight, preferably from 10 to 300% by weight, particularly preferably from 20 to 100% by weight, based on the active compounds.

The matrix present in the multicore structure is generally formed from a physiologically acceptable polymeric material. Preferably it is composed of at least one of the above-mentioned protective colloids, possibly in combination with the above-described formulation aids, such as plasticizers, antioxidants and/or emulsifiers. The matrix can also comprise at least one water-soluble vitamin.

The above-described solid preparations can be produced by drying an aqueous suspension comprising at least two active compounds which are suitable for the food sector and animal feed sector or for pharmaceutical and cosmetic applications in the form of nanoparticulate particles, which comprises at least two of the nanoparticulate particles having a different chemical composition. Active compounds here are the compounds already mentioned at the outset. In a preferred embodiment, the active compounds are at least two carotenoids, in which case, particularly preferably, at least two of the nanoparticulate particles comprise one or more different carotenoids.

For reasons of stability it is advantageous in this case if the active compounds are present in the form of protective-colloid-stabilized nanoparticulate particles which have a mean particle size of, preferably, from 0.01 to 1.0 μm, particularly preferably from 0.03 to 0.5 μm, very particularly preferably from 0.05 to 0.2 μm.

The active compounds, in particular the carotenoids, used to produce the inventive preparations can be used in the form of very finely ground crystals, or preferably in the form of preprepared dry powders. These dry powders each comprise nanoparticulate particles of the individual carotenoids and may be produced by grinding or micronizing individual active compounds. Examples of these may be found, inter alia, in EP-A-0 065 193, EP-A-0 937 412 and in WO 91/06292. By redispersing the starting formulations in aqueous solutions and converting the dispersion again into a dry powder by processes known per se, for example spray-drying or spray-cooling, with or without addition of dusting powders to avoid agglomeration, the novel inventive preparations having the multicore structures described at the outset may be obtained. Details on spray-drying or spray-cooling may be found, inter alia, in WO 91/06292.

The inventive carotenoid formulations are suitable, inter alia, as additives for food preparations, in particular drink preparations, as agent for producing pharmaceutical and cosmetic preparations and for producing food supplement preparations in the human and animal sectors. Thus, drinks may be fortified, for example, by using the inventive water-dispersible dry powders in which are present mixtures of beta-carotene, lycopene and lutein at the concentrations already mentioned above.

It is also possible to use dry powders which comprise the inventive carotenoid combinations to enrich milk products such as yogurt, flavored milk drinks or ice cream, or milk pudding powders, baking mixes and confectionery products, for example fruit gums.

The invention also relates to food supplements, animal feeds, foods and pharmaceutical and cosmetic preparations comprising the above-described preparations, in particular carotenoid formulations of mixtures of beta-carotene, lycopene and lutein. Food supplement preparations and pharmaceutical preparations which comprise the inventive dry powders are, inter alia, tablets, sugar-coated tablets and hard and soft gelatin capsules. Preferred food supplement preparations are tablets into which the dry powders are coincorporated, and soft gelatin capsules in which the carotenoid-containing multicore structures are present as oily suspension in the capsules. The carotenoid content in these capsules is from 0.5 to 20 mg of beta-carotene, from

0.5 to 20 mg of lycopene and 0.5 to 20 mg of lutein, preferably from 1 to 15 mg of beta-carotene, from 1 to 15 mg of lycopene and from 1 to 10 mg of lutein, particularly preferably from 2 to 10 mg of beta-carotene, from 2 to 10 mg of lycopene and from 1 to 5 mg of lutein.

Uses

Many disorders or diseases arise due to oxidative stress and the presence of free radicals. The methods of the present invention can be used to reduce, ameliorate, prevent, and/or treat disorders associated with antioxidant levels and excess free radicals. Populations at risk can be identified through methods known in the art (See, for example, US Patent Application No. 10/114,181 filed April 2, 2002,which describes an accurate, quick, and non-invasive method that can be easily adapted for high throughput usage and diagnostic procedures). At risk populations or people who wish to reduce the risk of free-radical associated disorders can benefit from the methods of the present invention. For example, disorders that can be reduced, ameliorated, prevented, and/or treated using the methods of this invention include, but are not limited to, aging at a higher than normal rate, segmental progeria disorders, Down's syndrome; heart and cardiovascular diseases such as arteriosclerosis, adriamycin cardiotoxicity, alcohol cardiomyopathy; gastrointestinal tract disorders such as inflammatory & immune injury, diabetes, pancreatitis, halogenated hydrocarbon liver injury; eye disorders such as cataractogenesis, degenerative retinal damage, macular degeneration; kidney disorders such as autoimmune nephrotic syndromes and heavy metal nephrotoxicity; skin disorders such as solar radiation, thermal injury, porphyria: nervous system disorders such as hyperbaric oxygen, Parkinson's disease, neuronal ceroid lipofuscinoses, Alzheimer's disease, muscular dystrophy and multiple sclerosis; lung disorders such as lung cancer, oxidant pollutants (O_3,NO_2), emphysema, bronchopulmonary dysphasia, asbestos carcinogenicity; red blood cell disorder such as malaria Sickle cell anemia, Fanconi's anemia and hemolytic anemia of prematurity; iron overload disorders such as idiopathic hemochromatosis, dietary iron overload and thalassemia; inflammatory-immune injury, for example, glomerulonephritis, autoimmune diseases, rheumatoid arthritis; ischemia reflow states disorders such as stroke and myocardial infarction; liver disorder such as alcohol-induced pathology and alcohol-induced iron overload injury; and other oxidative stress disorders such as AIDS, radiation-induced injuries (accidental and radiotherapy), general low-grade inflammatory disorders, organ transplantation, inflamed

rheumatoid joints and arrhythmias. The method of the invention can be used for reduction and prevention of a free radical induced disorder, or an oxidative stress disorder.

This invention is further illustrated by the following examples which should not be construed as limiting. The contents of all references, patents and published patent applications cited throughout this application, are incorporated herein by reference.

EXAMPLES

Subjects

Healthy female subjects (50-70 yr) were recruited for this study since post-menopausal women are generally more compliant and can avoid hormonal fluctuations. In order to minimize the possible variability of genetic differences, white females, not of Hispanic origin, were recruited from the general population and screened to select those with normal hematologic parameters, normal serum albumin, normal liver function, normal kidney function, absence of fat malabsorption and no drug intake which would interfere with fat absorption, metabolism or blood clotting. Subjects with a history of kidney stones, active small bowel disease or resection, atrophic gastritis, hyperlipidemia, insulin-requiring diabetes, alcoholism, pancreatic disease, or bleeding disorders were excluded from the study. Exogenous hormone users were also excluded from the study. Subjects weighing greater than 20% above or below the HANES median standard were excluded. Moreover, subjects were non-smokers and did not take vitamin or carotenoid supplements for at least 2 months prior to the study.

Study Design

Two weeks before starting the study (d-14), 10 mL of fasting blood was drawn from the subject as a check for basal levels of carotenoids, cholesterol, and triglycerides. Plasma pepsinogen was measured as a check for atrophic gastritis. Also, subjects were educated by a research dietitian to exclude foods rich in carotenoids (i.e. 2 servings of fruit and vegetable/day which is the average consumption in the U.S.) for two weeks prior to starting this study, and during study - except as provided by the Metabolic Research Unit (MRU) of the Human Nutrition Research Center on Aging (HNRC) at Tufts University. Three-day dietary records and

a Food Frequency Questionnaire were obtained 2 weeks prior to initiation of the study, as a check for carotenoid consumption.

Subjects (50-70 yr, n=40) were housed at the MRU for the first two days of the study. On the first day of the study, subjects were randomly assigned to take either 1) placebo, 2) 4 mg each of lutein, β-carotene and lycopene, 3) 4 mg of lutein, 4) 4 mg of β-carotene, or 5) 4 mg of lycopene with a meal containing 25g of fat. 10 mL of blood will be drawn at 0 (fasting), 2, 4, 6, 8, 10, 12 and 14 hours after the carotenoid dose to obtain information on the early kinetics of carotenoid absorption and tissue uptake. The subjects had the option to have an intravenous line inserted for blood drawing (I.V.). If they chose this option, 12 mL of blood was drawn for each sample and the first 2 mL of blood was discarded. Chylomicrons (the triglyceride-rich fraction of plasma) were isolated and analyzed for carotenoids to determine the plasma response kinetics in these 14 hr samples. Thereafter, subjects were discharged from the HNRC with a two-week supply of placebo or carotenoids along with instructions on how to consume the doses while being maintained on a low carotenoid diet. In particular, they were asked to take the carotenoid supplements with their major meal of the day.

From the second day of intervention, subjects took either 1) placebo, 2) 4 mg of lutein, 4 mg of β-carotene and 4 mg of lycopene, 3) 12 mg of lutein, 4) 12 mg of β-carotene, or 5) 12 mg of lycopene. On study days 15, 29, 43, and 57, overnight fasting bloods (10 mL) were collected, and 1) plasma carotenoids, 2) antioxidant capacity in both the aqueous and lipid compartments, 3) lipid peroxidation, and 4) DNA damage will be measured in these samples. In addition, 70 mL of fasting blood was collected at days 1 and 57 for the analysis of gene expression profiling in peripheral blood mononuclear cells using high-density filter-based cDNA microarrays. On study day 57, an additional 3 ml of blood was collected to measure the hemoglobin level.

Carotenoid supplements were provided to the volunteers on each sampling day while at the HNRC. The carotenoid supplements were supplied by BASF Corporation (Ludwigshafen, Germany). Dietary compliance was monitored by analyzing serum carotenoid concentrations,

counting remaining pills, and by evaluating three-day dietary records and a Food Frequency Questionnaire bi-weekly. The research dietician at the HNRC also interviewed study participants at each sampling day.

The total amount of blood collected for the study was 273 mL or 289 mL if drawn by I.V. A total of 273 mL or 289 mL of blood was drawn during the 8 wk period of entire study. The quantity of blood drawn has no known effects on health. Also, a study physician clinically reviewed the hemoglobin level of each subject at study day 57, and if needed, subjects were supplemented with iron. During blood drawing there is a small risk of bruising, bleeding or pain at the site of venous puncture. There is no known risk in taking supplemental carotenoids in the amounts given for this study. Four mg of lycopene is the amount that one absorbs from eating one medium tomato or 3/4 tablespoon of tomato paste, 4 mg of lutein is the amount that one absorbs from eating ~1/4 cup of cooked spinach, and 4 mg of beta-carotene is the amount that one absorbs from eating one and a third medium carrots or 1/4 cup of pumpkin. The low carotenoid diets (i.e. 2 servings of fruit and vegetable/day which is the average consumption in the U.S.) required prior to and during the study posed no risk to the subjects.

Analytical Techniques

Blood samples were protected from light and centrifuged within 1 h for 15 min at 1000 x *g* at 4°C, to separate plasma from red blood cells. Aliquots of plasma were stored at -70 °C until analyzed.

Measurement of antioxidant nutrients in plasma:

Plasma and chylomicron carotenoids were extracted using an enzyme extraction method, which gives 30-50% higher yield as compared to those of conventional extraction methods (Yeum et al *Am J Clin Nutr* 1996;64:594-602), and will be measured by an HPLC system with an Electrochemical Detector, which is ~10 times more sensitive than conventional photodiode array detector. Plasma concentrations of ascorbic acid (reduced form) and uric acid were determined by HPLC with an Electrochemical detector (ESA Inc., Bedford, MA).

Selective measurement of antioxidant capacity both in the lipid and aqueous compartments:

Aqueous and lipid plasma oxidation were induced at a constant rate by the azo-initiators: 1) AAPH as a water-soluble peroxyl radical generating system, 2) MeO-AMVN as lipid-soluble peroxyl radical initiators. Plasma oxidation were measured fluorimetrically using two different fluorescent probes: DCFH and C11-BODIPY 581/591 (BODIPY) (Aldini et al *Free Radic Biol Med.* 2001 Nov 1;31(9):1043-50).

Measurement of lipid peroxidation:

Lipid peroxidation was assessed by the measurement of malondialdehyde (MDA) using an HPLC system (Templar et al *Nephrol Dial Transplant* 2000;14:946-951). Also, F_2-isoprostanes will be measured using Mass Spectrometry (Morrow & Roberts *Methods Enzymol* 1999;3000:3-12).

Measurement of DNA oxidation:

DNA breaks and oxidized pyrimidine bases were measured using the alkaline comet assay (Duthie et al *Cancer Res* 1996;56:1291-1295). The comet assay, also called the Single Cell Gel Assay, was used to detect DNA damage and repair at the level of single cells. The Comet Assay is a rapid, sensitive test for DNA damage detection (e.g., single- and double-strand breaks, oxidative-induced base damage, and DNA-DNA/DNA-protein cross linking) by electrophoresis. The Comet Assay involves the following steps: 1. Slide preparation (i.e., mixing of cells with low melting agarose, and spread over glass microscope slides); 2. Lysis: (i.e., lysis of cell membrane and other proteins); 3. Unwinding of DNA; 4. Electrophoresis; 5. Neutralization; and 6. Staining and scoring. Cells embedded in agarose on a microscope slide are lysed with non-ionic detergent and high salt, leaving supercoiled matrix-attached DNA in a nucleoid. Under alkaline electrophoresis, DNA with breaks extends towards the anode, forming a "comet tail" when viewed by fluorescence microscopy. The percentage of total fluorescence in the tail is linearly related to DNA break frequency up to about 2 per 10^9 daltons.

Measurement of gene expression:

Gene expression profiling in peripheral blood mononuclear cells will be determined using high-density filter-based cDNA microarrays at University of Southern California at Los Angeles, CA (Walker & Rigley *J Immunol Methods* 2000;239:167-179).

Whole blood samples from 21 human volunteers were analyzed for carotenoid sensitive genes by quantitative analysis of mRNAs with GeneChips (HGU133A). The analysis detected the activities of 1500 to 6000 genes. The data from all the subjects were normalized and processed for clustering of genes with similar expressions (hierarchical clustering with D-Chip algorithm). The color-coded results correlated with the relative expression of genes. Genes that were differential affected in the 21 volunteers were "clustered" into functional classes using the D-chip algorithm. These classes are: 1) nucleic acid binding/ transcription factors; 2) ribosomal/ protein synthesis; 3) defense/immunity/antimicribial; 4) Major Histocompatibility Complex I and II; 5) stress response; 6) growth/maintenance; 7) metabolism; 8) wound healing; 9) inflammatory response. The genes in groups 3, 4, 8, and 9 may be different transcriptional responses to achieve the same biological end point of immune surveillance. These apparently diverse functional groups may be affected in a few cell types such as the lymphocytes and leukocytes where they may cooperate in immune surveillance. Regression analysis of a pair of data sets from the same volunteer can be performed; one data set of signal intensity may be obtained before intervention and the other after supplementation with carotenoids. Most of the genes are similarly expressed (< 3-fold change) in the volunteers. However, some genes show 3-30 fold differences in their expressions.

Genome wide analysis of human genes in whole blood samples has generated a list of genes that may be sensitive to dietary carotenoids. Functional classification of the differentially expressed genes suggests that genes that regulate immune surveillance may be targets of dietary carotenoids.

Statistics

At total sample size of 40 subjects (8 subjects per group) was used. The sample size was based upon the plasma carotenoid response data from a study using high fruit and vegetable diet (Yeum et al *Am J Clin Nutr* 1996;64:594-602), and from previous observations of plasma responses following carotenoid supplementation at doses similar to those in this study over 4 weeks. The sample size calculations were based on applying a logarithmic transformation to the data and were obtained by using the program PC-size (Dallal *Am Statistician* 1986;40:52) which implements methods from Snedecor and Cochran (Statistical Methods. 6th ed. The Iowa State University Press. Ames, IA, 1967) except that a non-central F distribution was used in the place of a non-central chi-squared distribution in order to accommodate smaller sample sizes. A sample of 8 subjects gave a 95% chance that a 0.05 level test of significance will find a statistically significant difference among the sample means. Results are expressed as means ± SEM and the significance of differences were determined by Student's *t* test or analysis of variance using the SYSTAT 9.1 (SPSS Inc., Chicago, IL). If the F statistic is significant ($p<0.05$), the Fisher least significance test was used to determine the differences between treatments at $p<0.05$ unless otherwise specified.

Results

The differential plasma appearance kinetics and functional bioavailability of lutein, β-carotene and lycopene in humans was studied. Information about the ability of a single carotenoid versus a combination of carotenoids to alter the entire antioxidant capacity (i.e. in both the lipid and aqueous compartments) of the circulation, affect the resistance of DNA to oxidation and gene expression in humans was obtained.

Resistance to oxidative stress in healthy women (n=37, 50-70 yr) in response to an 8-week period of carotenoid supplementation was determined. Subjects were randomly assigned to one of 5 groups to take a daily dose of either a combination of carotenoids (4 mg each of lutein, β-carotene and lycopene) or a single carotenoid (i.e., 12 mg each of lutein, β-carotene, or lycopene) for 8 weeks in a double-blind randomized trial. On study days 1, 15, 29, 43 and 57,

overnight fasting bloods were collected. See Figure 1 for a schematic illustration of the study framework. Figures 2-5 depict the alteration in plasma total carotenoid concentration, plasma lutein concentration, plasma beta-carotene concentration, and plasma lycopene concentration, respectively, following carotenoid supplementation. These results show that plasma carotenoid concentration can be modified by carotenoid supplementaion. Figure 6 compares the plasma carotenoid concentrations in elderly women (50-70 years old) of NHANES III (National Health and Nutrition Examination Survey) to that of the mixed carotenoid supplemented group at days 15 and 57 of this study. The results show that carotenoid supplemented group had plasma carotenoid concentrations similar to the seventy-fifth percentile of the NHANES III. Lymphocytes were separated immediately after blood collection and analyzed for oxidative DNA damage. A single cell gel electrophoresis (comet) assay was used to determine endogenous DNA damage, as well as hydrogen peroxide (H_2O_2 10 μM for 10 min) induced DNA damage. Figure 7 is an illustration of a sample comet assay showing the scoring system assigned to the staining patterns. The staining patterns were assigned a score from 0-4 where 0 indicated less than 5% DNA in the tail, 1 indicated 5-20% DNA in the tail, 2 indicated 20-40% DNA in the tail, 3 indicated 40-80% DNA in the tail, and 4 indicated greater than 80% DNA in the tail. The percent DNA break frequency is linearly related to total fluorescence in the tail. At least 100 cells were counted and scored in each sample.

All carotenoid supplemented groups showed significantly lower endogenous DNA damage at day 57 as compared to that of baseline ($p<0.005$), while the placebo group did not show any change. Figures 8 and 9 show that at day 57 the carotenoid supplemented group had a lower amount of DNA breaks (i.e., fewer scores greater than 0) than the placebo group.

In particular, the endogenous DNA damage in the mixed carotenoid supplemented group was significantly reduced after only 15 days ($p=0.002$) (See Figure 10 and Table 1). The effect of carotenoid supplementation on basal DNA damage was also compared with the placebo group as shown in Table 2. At all time points the mixture of carotenoids yielded a significant difference compared to the placebo group demonstrating that supplementation with the mixture of carotenoids led to a consistent, rapid effect.

When DNA was challenged with hydrogen peroxide (lymphocytes were treated with H_2O_2 at 10 micromolar for 10 min), DNA damage was significantly decreased at day 57 as compared to that of day 1 in the mixed carotenoid group, β-carotene group and lycopene group, while no change was observed in the placebo or lutein groups (See Figures 11 and 12 and Table 3). Figures 11 shows that at day 57 following mixed carotenoid supplementation less DNA was susceptible to oxidative damage by the hydrogen peroxide than at day 1. Figure 12 shows that DNA is less susceptible to oxidative damage by hydrogen peroxide following the mixed carotenoid supplementation compared to the placebo group. The results indicate that carotenoid supplementation can effectively protect against lymphocyte DNA damage and that the protective effect of mixed carotenoid supplementation against DNA damage is rapid and consistent. In addition, the protective effect of the physiologic dose of mixed carotenoid supplementation increased over time which indicates that the mixed carotenoid supplementation has a cumulative positive effect on the subjects.

Table 1. Effect of Carotenoid Supplementation on Basal DNA Damage (Comet tail factor, %)
Note: Bold numbers indicate significant difference from Day 1 ($p<0.05$)
Unit: Mean ± SD

Group	D1	D15	D29	D43	D57
Placebo (n=6)	8.7 ± 2.0	9.1 ± 2.5	10.6 ± 3.2	9.2 ± 4.1	9.9 ± 3.8
Mixed CAR (n=8)	10.9 ±1.5	**8.6 ± 1.6**	**7.9 ± 1.8**	**7.1 ± 1.4**	**7.0 ± 1.3**
Lutein (n=8)	10.6 ±1.4	9.4 ± 2.1	9.5 ± 1.4	**7.7 ± 1.5**	**7.1 ± 1.7**
β-Carotene (n=7)	12.4 ±2.7	**9.7 ± 2.4**	**8.6 ± 3.0**	**9.4 ± 2.4**	**8.0 ± 1.9**
Lycopene (n=8)	11.9 ±2.6	**10.0 ± 3.5**	**9.1 ± 2.6**	**7.5 ± 1.9**	**6.8 ± 1.6**

Table 2. Effect of Carotenoid Supplementation on Basal DNA Damage (Compared with Placebo Group, t-test)
Note: Bold numbers indicate significant difference from Day 1 ($p<0.05$)
Unit: P-value

	D15 / D1	D 29 / D1	D43 / D1	D57 / D1
Mixed Car	**0. 021**	**0. 017**	**0. 018**	**0. 010**
Lutein	0. 255	0. 061	**0. 047**	**0. 011**
β-Carotene	**0. 014**	**0. 021**	0. 127	**0. 018**
Lycopene	0. 059	**0. 014**	**0. 019**	**0. 004**

Table 3. Effect of Carotenoid Supplementation on DNA Susceptibility Against Oxidative Damage (Comet tail factor, %)
Note: Lymphocytes were treated with H_2O_2 (10 μM for 10 min)
Bold numbers indicate significant difference from Day 1 ($p<0.05$)
Unit: Mean ± SD

	Dav1	Dav15	Dav29	Dav43	Dav57
Placebo (n=6)	42.1±5.4	44.6±6.0	39.7±2.8	43.0±7.1	40.6±7.6
Mixed Car (n=8)	44.2±7.0	43.2±9.2	42.6±7.7	37.1±11.3	**36.4±6.2**
Lutein (n=8)	42.8±6.8	43.5±6.2	43.1±5.6	41.5±9.3	39.8±8.4
β-Carotene (n=7)	48.2±6.1	44.5±8.9	41.1±6.3	44.2±5.7	**38.0±4.8**
Lycopene (n=8)	50.6±8.9	49.2±10.1	51.1±3.5	50.1±6.5	**42.5±6.5**

Claims:

1. A method of modulating oxidative DNA damage in a subject comprising:

administering an effective dose of at least two of the group consisting of substantially purified lutein, beta-carotene, and lycopene.

2. The method of claim 1, wherein the effective dose is selected from about 0.5 mg to 20 mg beta-carotene, about 0.5 to 20 mg lycopene, and about 0.5 to 20 mg lutein.

3. A method of decreasing oxidative damage in a subject comprising:

administering a synergistic combination of carotenoids to the subject, wherein the synergistic combination comprises at least two of the group consisting of lutein, beta-carotene, and lycopene.

4. The method of claim 3, wherein the method comprises administering about 0.5 mg to 20 mg beta-carotene, about 0.5 to 20 mg lycopene, and about 0.5 to 20 mg lutein to the subject.

5. The method of claim 3, wherein the method comprises administering about 1 mg to 10 mg beta-carotene, about 1 to 10 mg lycopene, and about 1 to 10 mg lutein to the subject.

6. The method of claim 3, wherein the method further comprises administering the synergistic combination with a lipophilic component.

7. A method of protecting against a free radical associated disorder comprising:

administering a synergistic combination of carotenoids to the subject, wherein the synergistic combination comprises at least two of the group consisting of lutein, beta-carotene, and lycopene.

ABSTRACT

The methods of the invention can be used to protect against lymphocyte DNA damage and free-radical associated disorders in a subject. The methods of the present invention can be used to increase the antioxidant capacity in one or both of the aqueous and lipid compartments, decrease DNA oxidation, increase gene expression of a panel of genes affected by carotenoids, decrease lipid peroxidation, or increase antioxidant nutrient levels in the circulation. The protective effect of a physiologic dose of a mixed carotenoid supplement, according to the invention, is rapid, consistent and cumulative.

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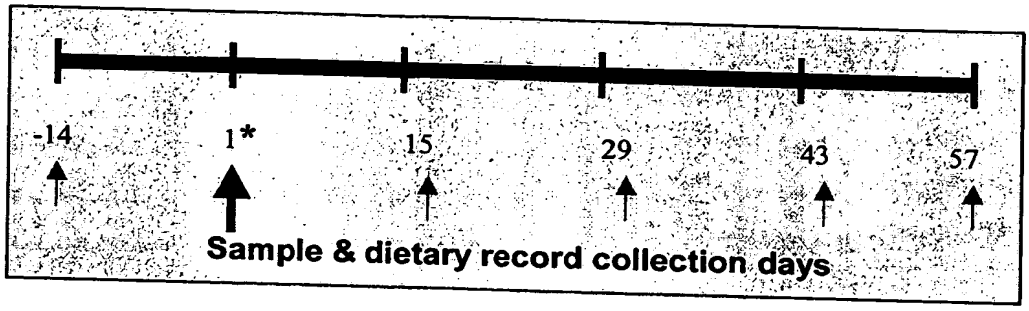
FIGURE 1

Study Framework

Study groups

Group 1 (n=8): placebo
Group 2 (n=8): lutein, 4 mg; β-carotene, 4 mg; & lycopene, 4 mg
Group 3 (n=8): lutein, 12 mg
Group 4 (n=8): β-carotene, 12 mg
Group 5 (n=8): lycopene, 12 mg



Key:
↑ Fasting blood
↑ 0, 2, 4, 6, 8, 10, 12, & 14 hr blood to determine carotenoid appearance kinetics
→ chylomicrons will be isolated & analyzed for carotenoids

* Subjects in groups 3-5 will take 4 mg carotenoid in day 1, thereafter will take 12 mg carotenoid

Day 1: Group 3: lutein, 4 mg; Group 4: β-carotene, 4 mg; Group 5: lycopene, 4 mg

Days 2-56: Group 3: lutein, 12 mg; Group 4: β-carotene, 12 mg; Group 5: lycopene, 12 mg

Plasma sample analysis:

- Oxidizability (Lipid & Aqueous compartments)
- Lipid peroxidation (MDA-TBA Adducts)
- Lipid peroxidation (Isoprostane)
- DNA damage (Comet assay)
- Lutein, β-carotene, lycopene levels
- α-Tocopherol, ascorbic acid, uric acid levels
- Gene expression (high-density filter-based cDNA microarrays)

Three-day dietary records & FFQ: bi-weekly

FIGURE 2

Alteration of Plasma Total Carotenoid Concentration By Carotenoid Supplementation

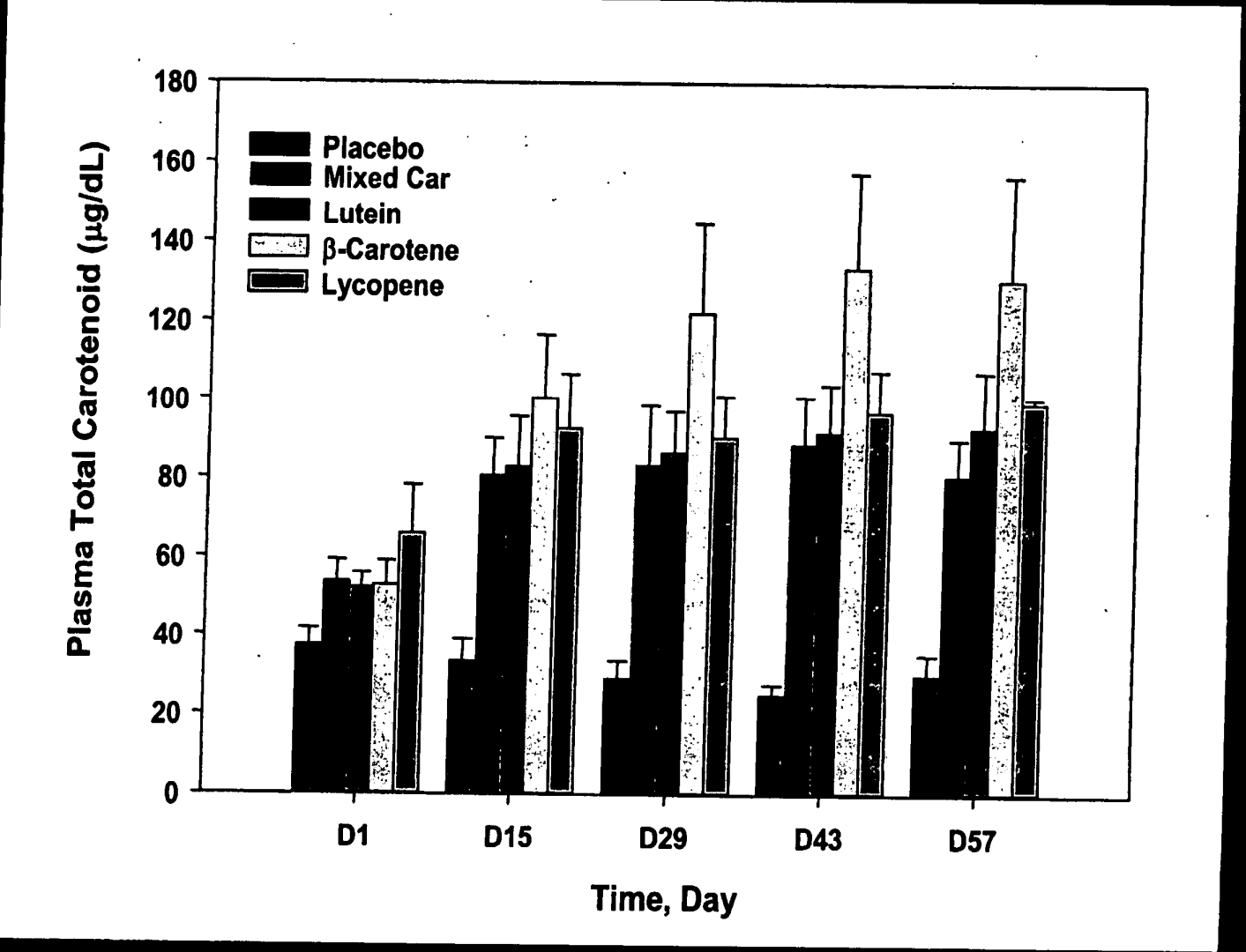


FIGURE 3

Alteration of Plasma Lutein by Carotenoid Supplementation

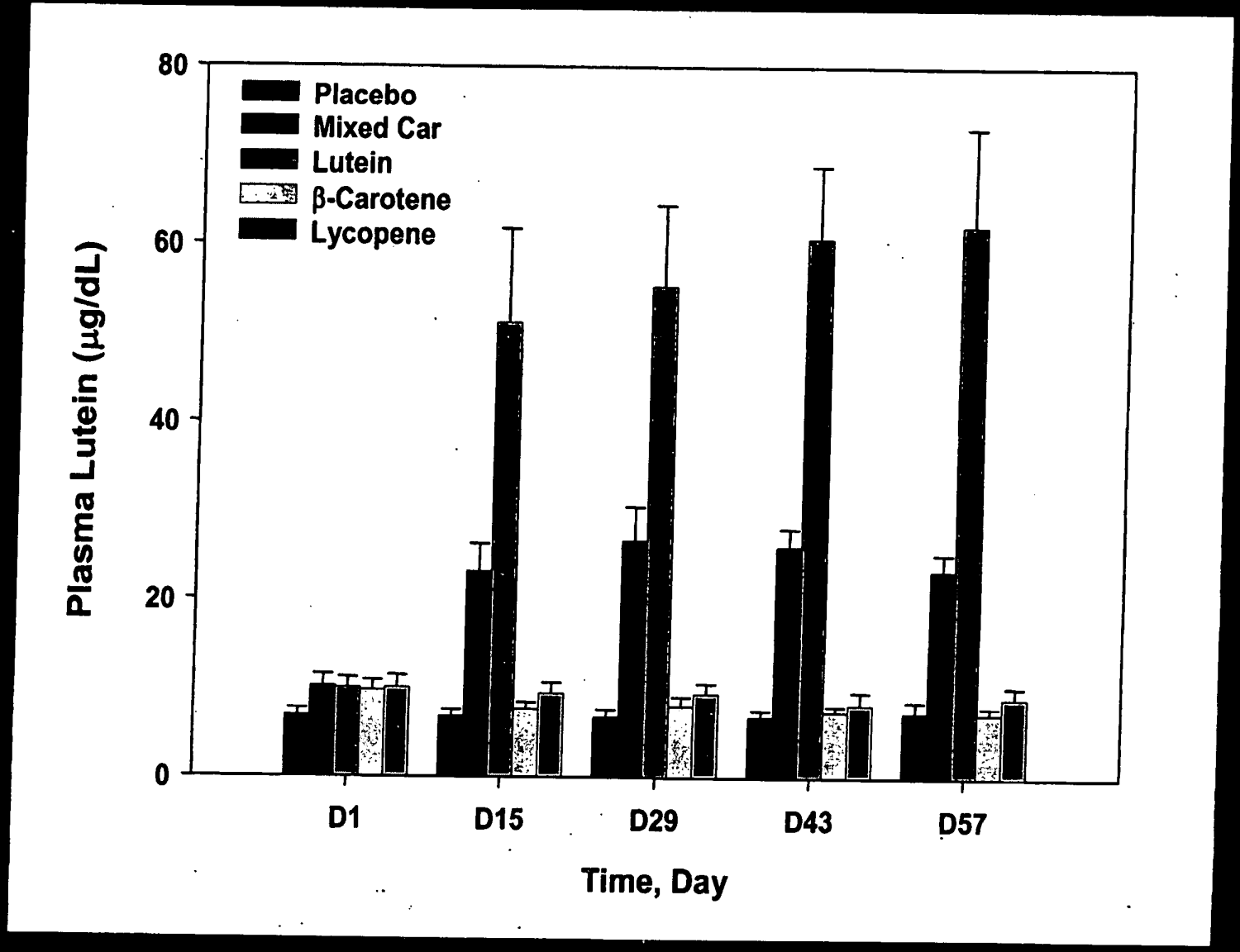


FIGURE 4

Alteration of Plasma β-Carotene By Carotenoid Supplementation

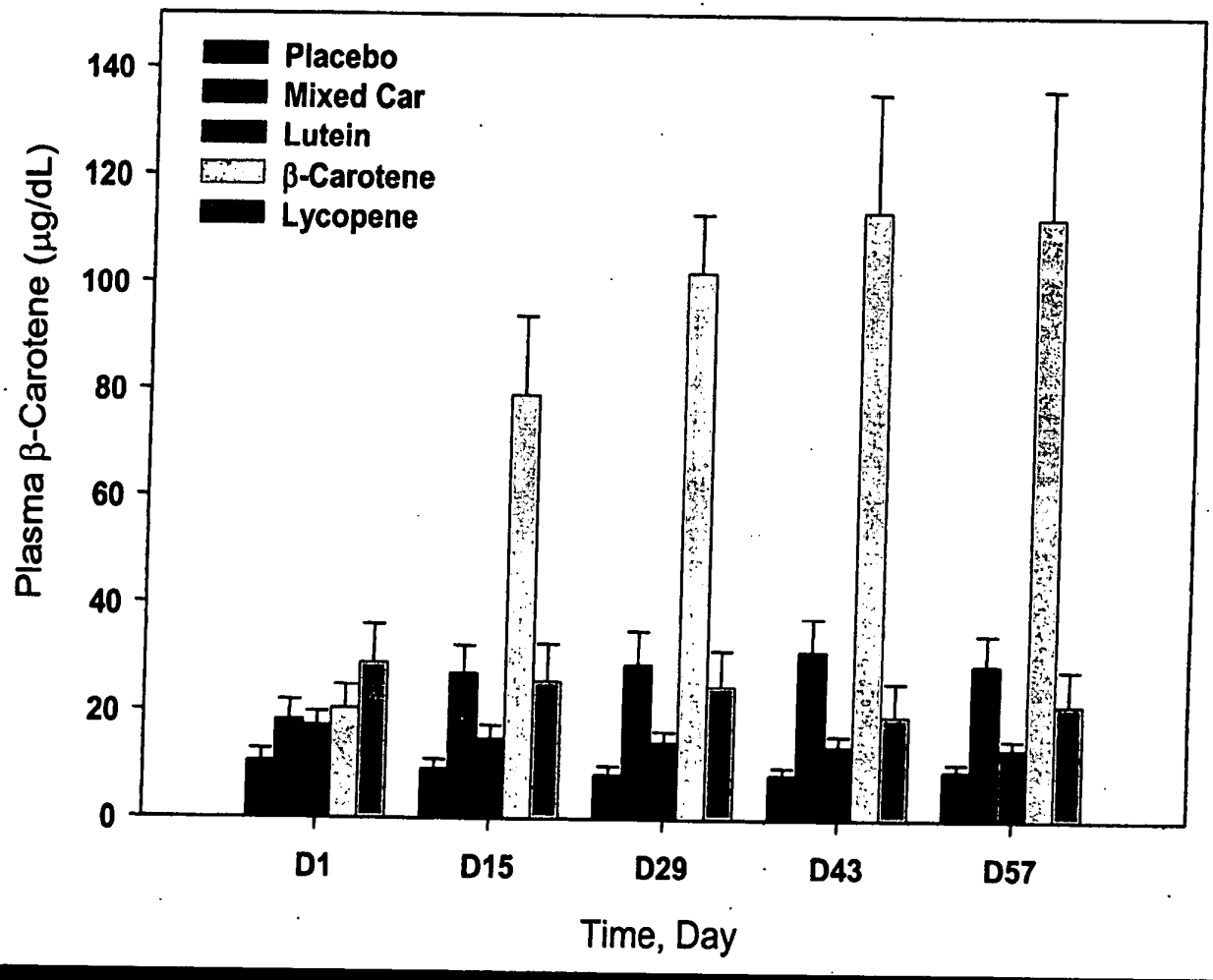


FIGURE 5

Alteration of Plasma Lycopene By Carotenoid Supplementation

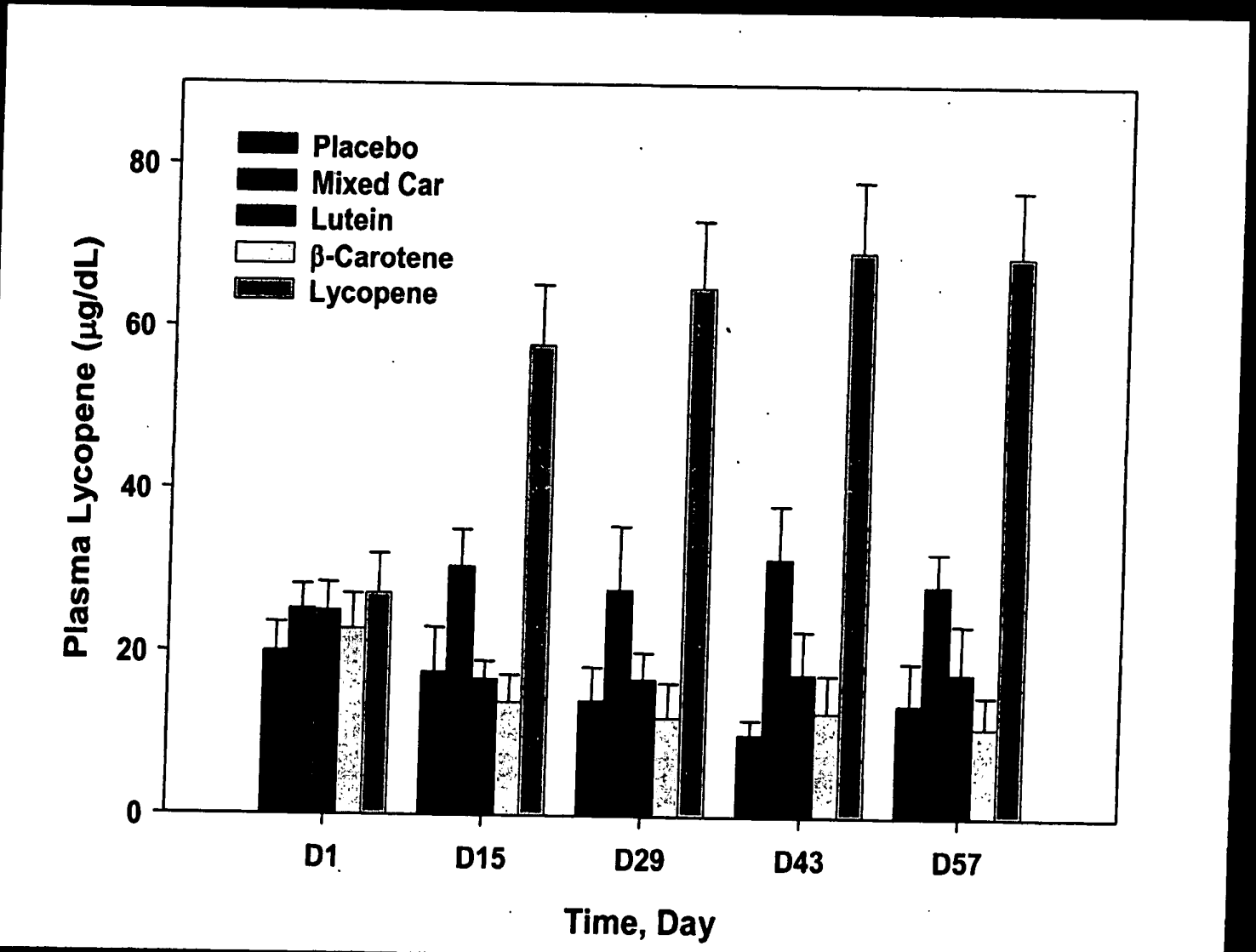


FIGURE 6

Plasma Carotenoid Concentrations in Elderly Women (50-70 yr) of NHANES III And in Mixed Carotenoid Supplemented Group

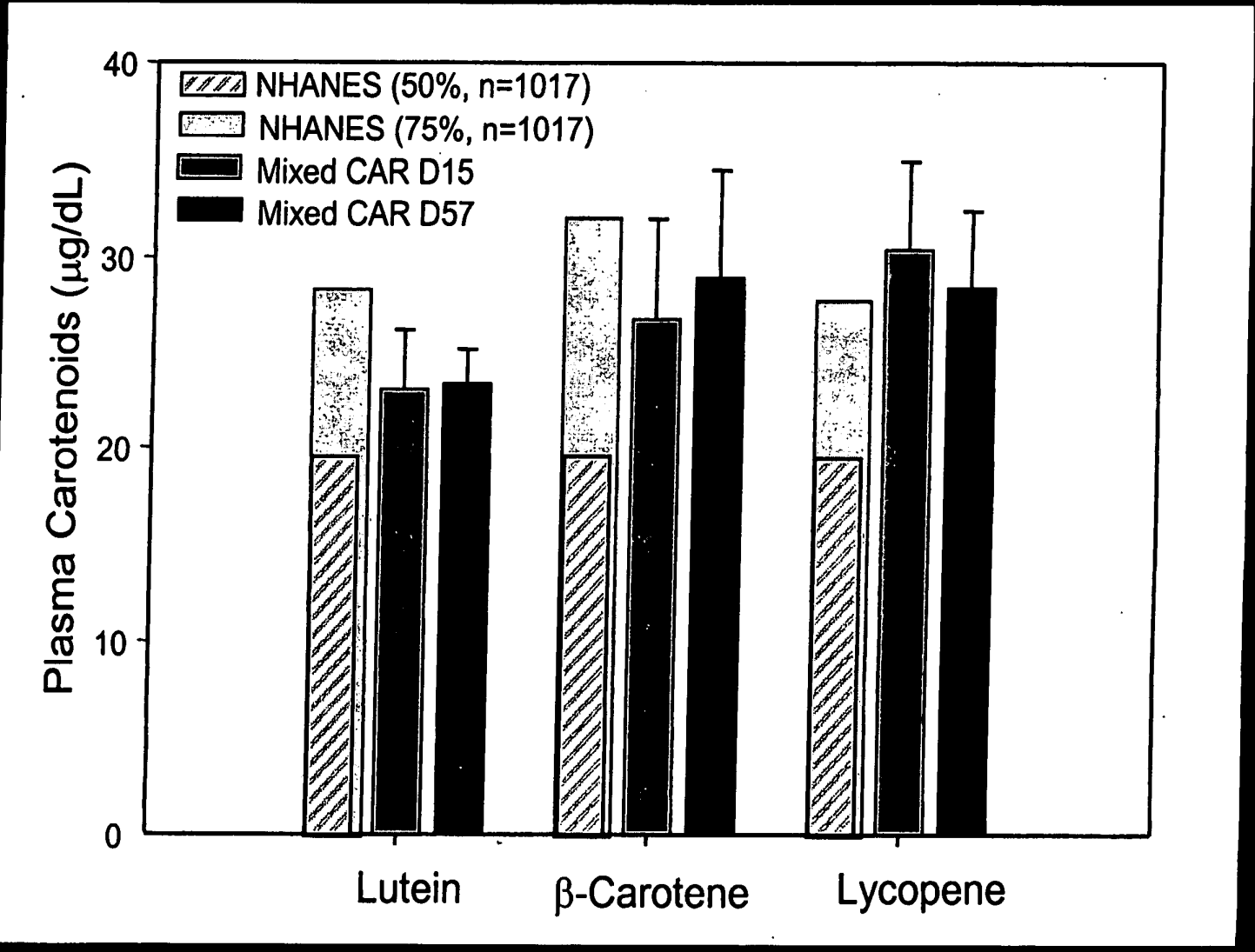
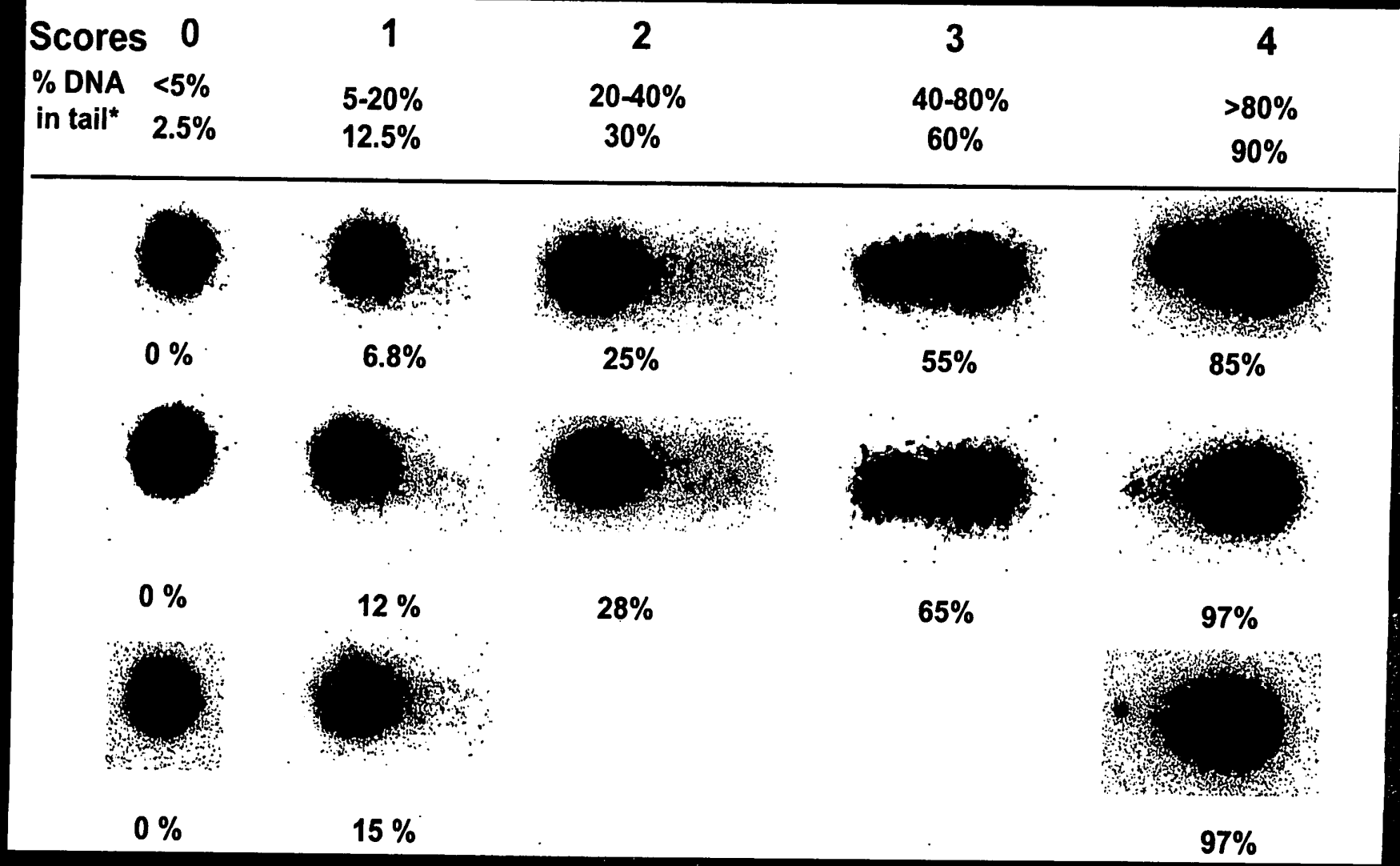


FIGURE 7

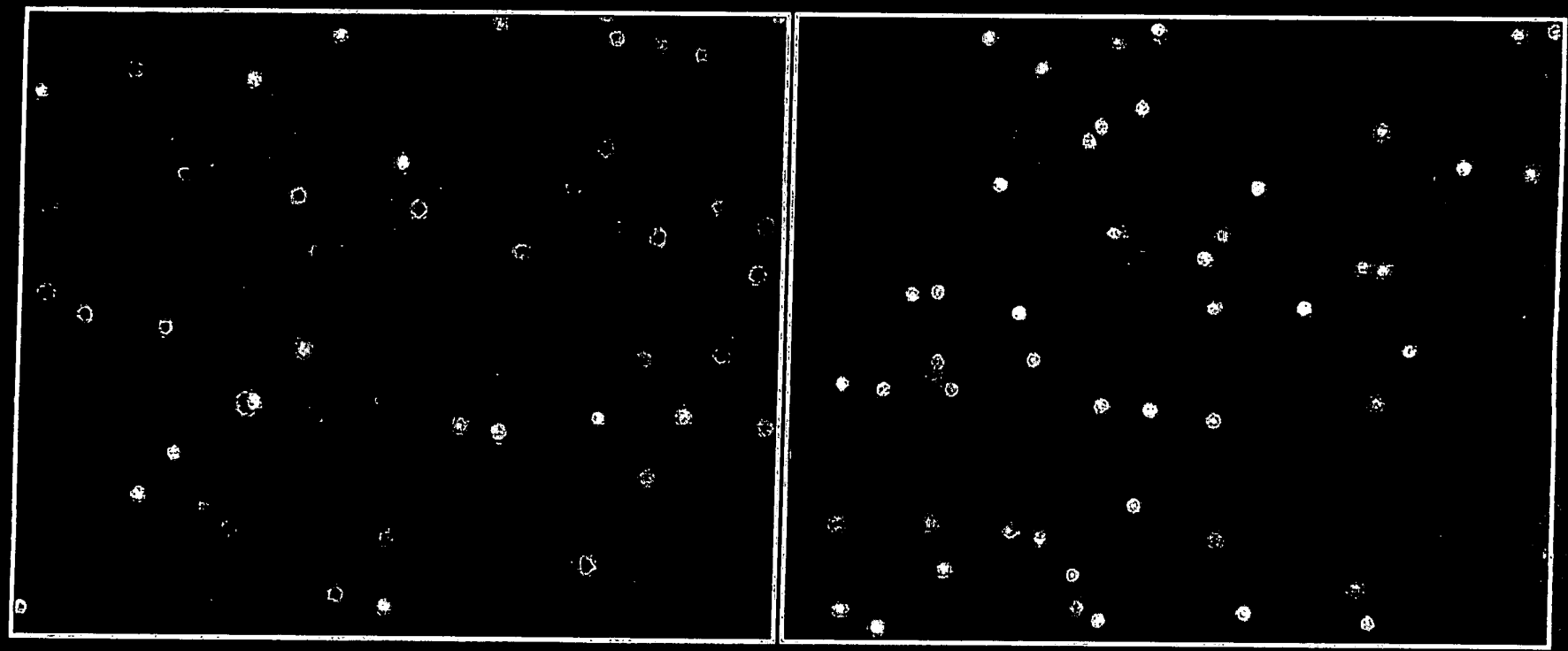
Quantitation of DNA damage: Visual analysis



At least 100 cells were counted & scored in each sample.

FIGURE 8

Effect of Carotenoid supplementation on Basal DNA damage



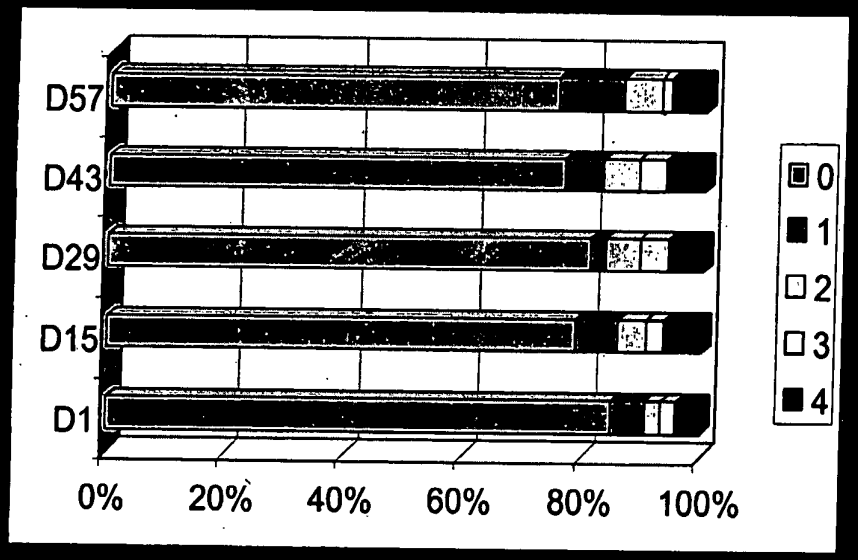
Day 1

Day 57

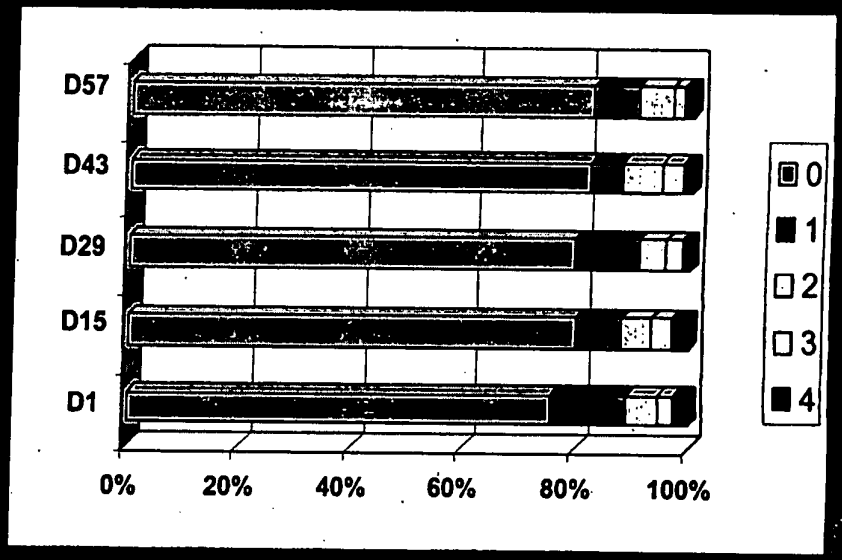
(Subject # 35563)

FIGURE 9

Effect of Carotenoid supplementation on Basal DNA damage



Placebo
(Subject # 24710)



Carotenoid
(Subject # 34865)

FIGURE 10

Effect of Carotenoid Supplementation on Basal DNA damage

Comet tail factor ratio compared with day1

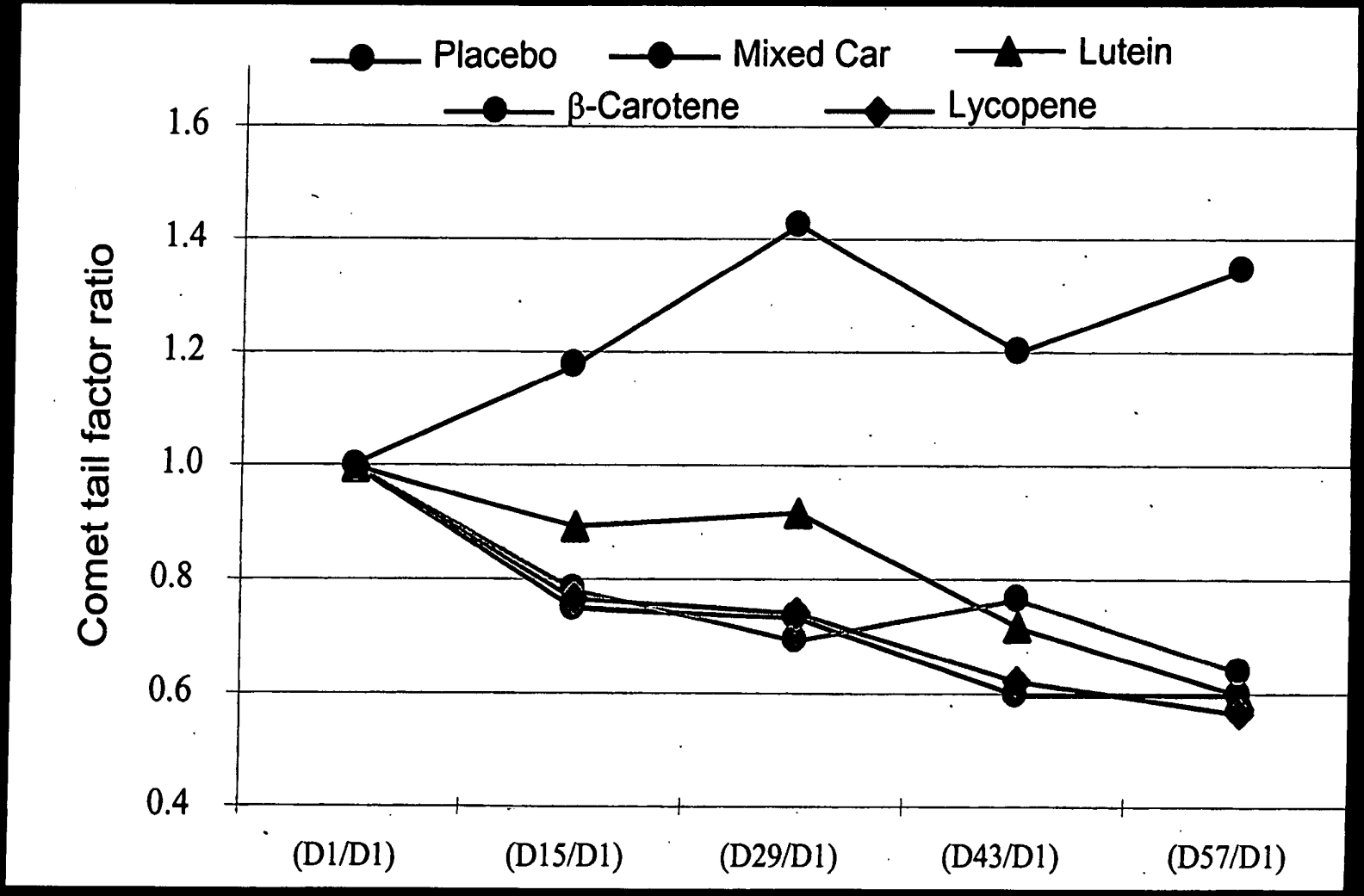
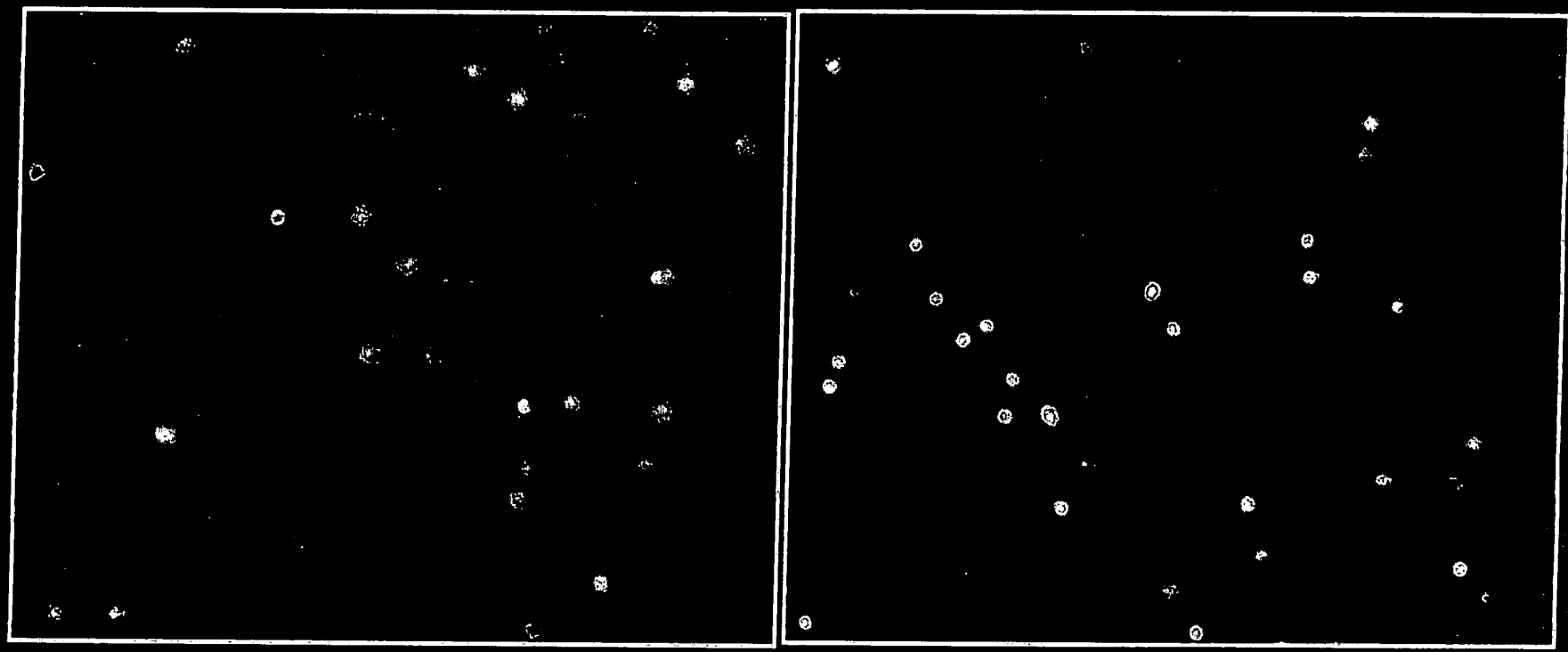


FIGURE 11

Effect of Carotenoid supplementation On DNA Susceptibility Against Oxidative Damage*



Day 1

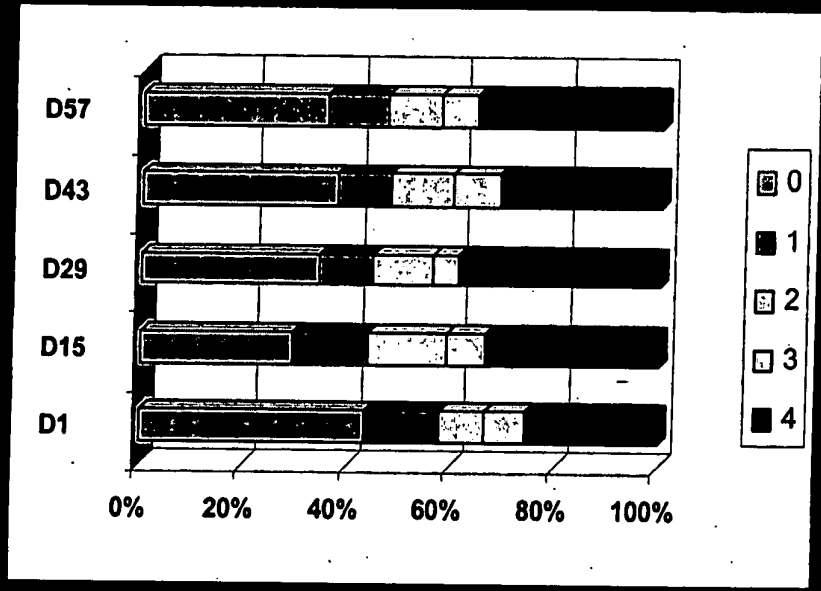
Day 57

Subject # 34884

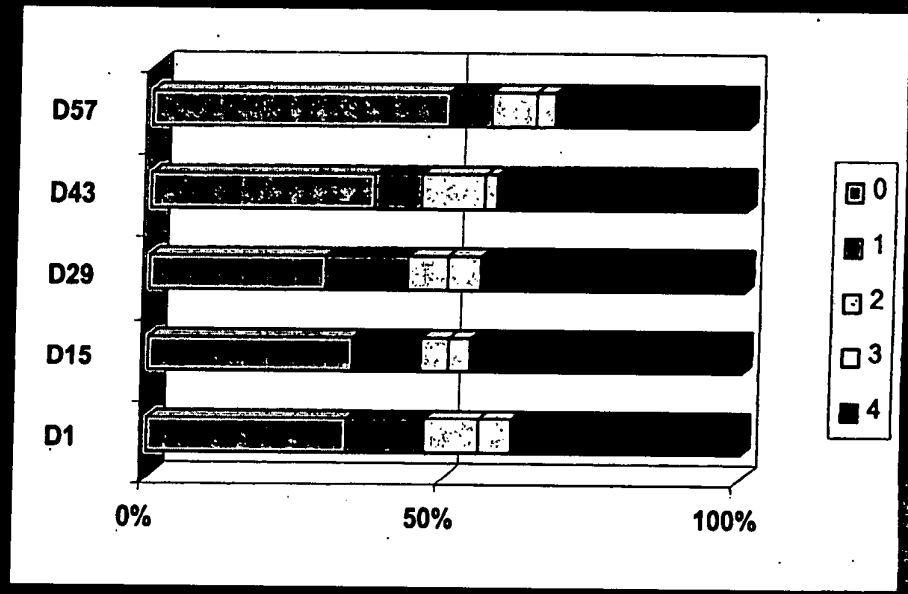
*10 μM H_2O_2 for 10 min

FIGURE 12

Effect of Carotenoid supplementation On DNA Susceptibility Against Oxidative Damage*



Placebo
(Subject # 27121)



Carotenoid
(Subject # 34865)

*10 μM H_2O_2 for 10 min

Application Data Sheet

Application Information

Application number:	Not Yet Assigned
Application Type:	Regular
Subject Matter:	Provisional
Title:	Bioavailability of Carotenoids
Attorney Docket Number:	5363-3226
Small Entity:	Yes
Secrecy Order in Parent Appl.:	No

Applicant Information

Applicant Authority Type:	Inventor
Citizenship Country:	Korea
Given Name:	Kyung-Jin
Family Name:	Yeum
City of Residence:	Winchester
State of Residence:	Massachusetts
Street of mailing address:	29 Lockeland Road
City of mailing address:	Winchester
State or Province of mailing address:	MA
Country of mailing address:	US
Postal or Zip Code of mailing address:	01890

Correspondence Information

Correspondence Customer Number:	021125
Phone number:	(617) 439-3948
Fax number:	(617) 310-9948
E-Mail address:	tengellenner@Nutter.com

Representative Information

Representative Customer Number:	021125

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